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30. (New) A mammary gland epithelial cell comprising the construct of claim 28 and a construct comprising an immunoglobin protein-coding sequence which encodes a light chain or a fragment thereof, operatively linked to a promoter sequence that results in the preferential expression of the protein-coding sequence in mammary gland epithelial cells, wherein the cell expresses the light and heavy chains and secretes a heterologous, assembled immunoglobin comprising the light and heavy chains in functional form.

REMARKS

Claims 19-30 are pending. Claims 19 and 22 have been amended. Claims 20 and 24 have been canceled without prejudice. However, the amendment to and/or cancellation of the claims have been made solely to expedite prosecution of the present application. New claims 26-30 have been added. Support for new claims 26-30 can be found throughout the specification of the application as originally filed. No new matter has been added.

Rejection of Claims 19 and 21-25 Under 35 U.S.C. §112, first paragraph

Claims 19 and 21-25 are rejected under 35 U.S.C. §112, first paragraph, "because the specification, while being enabling for generating a construct comprising a tissue-specific promoter operably linked to a sequence encoding an immunoglobulin, does not reasonably provide enablement for using the sequence to generate any transgenic animal harboring the construct." In particular, the Examiner states that

> While the specification is enabling for a DNA construct comprising a mammary tissue-specific promoter and sequences encoding heavy and light chains of immunoglobulins, the specification is non- enabling for the expression and secretion of an assembled immunoglobin into the milk of a transgenic mammals per se.
>
> The intended use of the DNA construct is to generate transgenic mammals which express the transgene in the mammary gland epithelial cells such that the immunoglobulins are secreted, in an assembled form, into the milk of a lactating transgenic mammal. The generation of constructs comprising "mammary tissue-specific" promoters such as the casein, lactoglobulin, lactoalbumin, and whey protein promoters, to direct expression of a sequence encoding a foreign protein into the milk of transgenic mammals such as cows, goats, sheep, and mice is well known in the art (see, e.g., Table 6 in Houdebine, Journal of Biotechnology,

34:269-287). However, the state of the art at the time of filing is such that the generation of transgenic mammals other than mice, via embryonic stem cell technology, is neither routine nor predictable. For example, Bradley et al. state that the key requirement in any experiment involving the generation of a specific modification in ES cells is that the clone should retain all of its potential to contribute to both somatic lineages and the germ line following microinjection of the cells into blastocyst-stage embryos (see pages 535-536, bridg. Sentence in Bradley et al., Bio/Technology, 10:534-539, 1992). Bradley et al. disclose that at that time, there were no ES cells for any animal other than mouse which had been established to give rise to somatic tissues or germ cells in vivo. Similarly, Seamark (Reproductive Fertility and Development, 6:653-657, 1994) discloses that totipotency for ES cell technology in many livestock species has not been demonstrated (see, e.g., Abstract). Moreover, Mullins et al. teach that while chimeric animals for several species had been produced using purported ES cells, germ line transmission of an ES cell has not been demonstrated in species other than mice. In addition, Mullins et al disclose that "The use of nonmurine species for transgenesis will continue to reflect the suitability of a particular species for the specific questions being addressed, bearing in mind that a given construct may react very differently from one species to another" (see page S37-S38, bridg. Sentence, page S38, col. 1, lines 23-26, and page S39, under "summary" in Mullins et al., J. Clin. Invest., 98:S37-S40, 1996). In view of the state of the art at the time of filing, the generation of a transgenic mammal from any species, which is an essential element for the implementation of the claims, was not readily available to a skilled artisan either through the art or by the disclosure in the specification.

Applicants respectfully traverse this rejection. The claims, as amended, are directed to a DNA construct for providing a heterologous immunoglobin in the milk of a transgenic mammal. The construct includes a promoter sequence that results in the preferential expression of a protein-coding sequence in mammary gland epithelial cells, an immunoglobin protein-coding sequence, a 3' non-coding sequence, and a unique restriction site between the promoter and the 3'non-coding sequence, wherein the immunoglobin protein-coding sequence is inserted into the restriction site. In addition, the claims are directed to a cell which includes such constructs and which expresses the light and heavy chains and secretes a heterologous, assembled immunoglobin comprising the light and heavy chains.

As acknowledged by the Examiner, there is sufficient guidance in the present application to make the DNA construct as claimed. Thus, the claimed invention is enabled.

In addition, Applicants disagree with the Examiner's assertions regarding the state of the art of transgenic technology. The Examiner asserts that "the state of the art at the time of filing is such that generation of transgenic animals other than mice, via embryonic stem cell technology, is neither routine nor predictable" and relies on several references which discuss the production of transgenic mammals via embryonic stem cell technology. However, other methods, such as microinjection which is described in the present application, were known and had been used to produce transgenic mammals other than mice. For example, Hammer et al. "Production of Transgenic Rabbits, Sheep and Pigs By Microinjection" (1985) *Nature* 315:680-683 (submitted herewith as Exhibit A), describe the production of a variety of transgenic species by microinjection. In addition, Kraemer et al. *Gene Transfer into the Pronuclei of Cattle and Sheep Zygotes,* pp.221-222 (1985) (submitted herewith as Exhibit B) provides techniques which were used to make with transgenic cows. Both of these references were published before the effective filing date of this application. References such as these provide comprehensive and detailed guidelines for the microinjection of foreign DNA into a variety of mammals and prove that the production of such transgenic mammals is enabled.

For the reasons discussed above, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims 19-25 Under 235 U.S.C. §112, second paragraph

Claims 19-25 are rejected under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In particular, the examiner states that claim 19 "is render vague and indefinite by the phrase 'heterologous immunoglobin protein-coding sequence' as it is unclear if the sequence is heterologous to the promoter sequence, to the transgenic mammal or both."

Applicants have amended the claims. As amended, the claims no longer recite the phrase "heterologous immunoglobin protein-coding sequence".

The Examiner also states that claim 19 "is further rendered vague and indefinite by the phrase "preferential expression" as it is unclear if the expression of the protein is tissue-

restricted, tissue-specific, expressed during a certain time of development, expressed under certain physiological conditions, etc. Thus, the metes and bounds are unclear."

Applicants respectfully traverse this rejection. At page 6 of the specification, Applicants state that "promoters useful in practicing the present invention are those promoters that are preferentially activated in mammary epithelial cells, including promoters that control the genes encoding milk proteins such as caseins, beta lactoglobulin . . . whey acid protein . . . and lactoalbumin." Based on the definition in the specification, the meaning of the term "preferential expression is clear. Therefore, Applicants request that the Examiner withdraw this rejection.

In addition, the Examiner states that "claim 19 is also confusing by the functional language utilized in describing the construct." According to the Examiner,

> while promoters are known to 'direct' expression of a gene, and while constructs can be expressed in the mammary gland epithelial cells in a mammal harboring the construct such that a foreign protein can be secreted into the milk of a transgenic animal, it is unclear how a promoter, per se, can 'result in' preferential expression of the protein coding sequence or 'thereby' provide a heterologous and assembled immunoglobin in the milk of a transgenic animal. Moreover, in light of the specification which describes two distinct DNA constructs which separately encode a heavy or a light chain, the claim is confusing because it recites "A DNA construct comprising a heterologous immunoglobin protein coding sequence" which when expressed, is assembled. It is unclear what protein-coding sequence is required in the heterologous immunoglobin protein-coding sequence such that the immunoglobin is secreted into milk in an assembled form, are unclear.

Claim 19 has been amended, thereby obviating this rejection.

Rejection of Claims 19-21, 24 and 25 Under 35 U.S.C. §102

Claims 19-21, 24 and 25 are rejected under 35 U.S.C. §102(b) as being anticipated by Meade et al. (U.S. Patent No. 4,873,316) or under 102(e) as being anticipated by Meade et al. (U.S. Patent No. 5,750,172). The Examiner applies the same assertions for both the Meade et al. '316 patent and Meade et al.'172 patent, thus both the 102(b) and 102(e) rejections are addressed below. According to the Examiner,

> Meade *et al.* disclose a DNA construct for production of recombinant proteins comprising a milk-specific protein promoter or any promoter sequence specifically activated in mammary tissue, operatively linked to a DNA sequence coding for a desired recombinant protein through a DNA sequence coding for a

signal peptide that permits the secretion and maturation of the desired recombinant protein in the mammary tissue. The construct can be transgenically incorporated into mammalian embryos obtained from cows, sheep, goats, mice, and pigs, for example, such that the recombinant protein product is subsequently expressed and secreted into or along with the milk of the lactating transgenic animal (see, e.g., column 2, lines 41-68). The milk-specific protein promoter or promoter sequence specifically activated in mammary tissue can be selected from the casein promoters, β-lactoglobulin promoter, or the long terminal repeat promoter of the mouse mammary tumor virus (see, e.g., column 3, lines 1-15). The DNA sequence coding for a desired recombinant protein can include sequences encoding immunoglobulins (see, e.g., column 3, lines 30-40).

Thus, DNA constructs comprising a promoter sequence operatively linked to a sequence encoding immunoglobulins, wherein the promoter is preferentially expresses the immunoglobulins in the mammary gland epithelial cells of transgenic mammals such as cows, sheep, goats, mice, and pigs, and is secreted into the milk of the mammal, are anticipated by Meade *et al.*

Applicants respectfully traverse this rejection. As amended, the claims are directed to a DNA construct for providing a heterologous immunoglobin in the milk of a transgenic mammal. The construct includes a promoter sequence that results in the preferential expression of a protein-coding sequence in mammary gland epithelial cells, an immunoglobin protein-coding sequence, a 3' non-coding sequence, and a unique restriction site between the promoter and the 3'non-coding sequence, wherein the immunoglobin protein-coding sequence is inserted into the restriction site. In addition, the claims are directed to a mammary gland epithelial cell which includes at least two of the claimed constructs for producing a heavy and a light chain, wherein the cell expresses the light and heavy chains and secretes a heterologous, assembled immunoglobin comprising the light and heavy chains.

Neither Meade et al. '316 patent nor the Meade et al. '172 patents teach or suggest the claimed construct having a unique restriction site in between the promoter and the 3' untranslated region into which an immunoglobin protein-encoding sequence is inserted. Thus, neither Meade et al. '316 nor Meade et al. '172 teach or suggest every element of the claimed invention, and therefore these references do not anticipate the claimed invention.

Rejection of Claims 19-25 Under 35 U.S.C. §103(a)

Claims 19-25 are rejected under 35 U.S.C. §103(a) as being unpatentable over Meade et al. (U.S. Patent No. 4,873,316), taken with Bischoff et al. (1992) FEBS Letters 305:265-268; Buhler et al. (1991) Bio/Technology 9:835-838; Gordon et al. (1987) Bio/Technology 5:1183-1187; Ebert et al. (1990) Bio/Technology 8:140-143; or Stinnakre et al. (1991) FEBS Letters 284:19-22, and further in view of Boss et al. (U.S. Patent No. 4,816,397), Bruggeman et al. (WO 90/04036), and Weidle et al. (1991) Gene 98:185-191. In particular, the Examiner states

> Meade et al disclose a DNA construct for the production of recombinant proteins comprising milk-specific protein promoter or any promoter sequence specifically activated in mammary tissue, operatively linked to a DNA sequence coding for a desired recombinant protein through a DNA sequence coding for a signal peptide that permits the secretion and maturation of the desired recombinant protein in the mammary tissue. The construct can be transgenically incorporated into mammalian embryos obtained from cows, sheep, goats, mice, and pigs, for example, such that the recombinant protein product is subsequently expressed and secreted into or along with the milk of the lactating transgenic animal (see, e.g., column 2, lines 41-68). The milk-specific protein promoter or promoter sequence specifically activated in mammary tissue can be selected from the casein promoters, β-lactoglobulin promoter, or the long terminal repeat promoter of the mouse mammary tumor virus (see, e.g., column 3, lines 1-15). The DNA sequence coding for a desired recombinant protein can include sequences encoding immunoglobulins (see, e.g., column 3, lines 30-40).
>
> Meade et al. do not disclose that the promoter can be selected from whey acid protein promoter or the lactalbumin promoter, that the immunoglobin comprises heavy and light chains, or that the immunoglobin is of human origin.
>
> With regard to the claim-designated promoter sequences, Bischoff et al. disclose a construct containing a sequence encoding human α1-antitrypsin variant operatively linked to 17.6 kb of rabbit whey acid protein promoter, which results in the expression and secretion of the α1-antitrypsin variant into the milk of a transgenic mouse . . . Similarly, Gordon et al. disclose a DNA construct containing a sequence encoding human tissue plasminogen activator activator (t-PA) operatively linked to the promoter and upstream regulatory sequence from the murine whey acid protein gene, which results in the expression of t-PA into the milk of a transgenic mouse . . . In addition, Elbert et al. disclose a DNA construct containing a sequence encoding human tissue plasminogen activator operatively linked to the mouse whey acid protein promoter which results in expression of the protein into goat milk . . . moreover, Stinnakre et al. disclose a DNA construct comprising a sequence encoding ovine trophoblast interferon operatively linked to the promoter of the bovine α-lactalbumin gene, wherein the

construct is capable of being expressed in the mammary gland of mice and secreted into milk . . . From the teachings of Bischoff et al., Gordon et al., Elbert et al., or Stinnakre et al., one of ordinary skill in the art would have had a high expectation of successfully producing a protein by the mammary gland which is secreted into the milk of a mammal using a DNA construct which contains a whey acid protein promoter or a lactalbumin promoter, which is known in the art to direct expression of the foreign protein in the mammary gland.

With regard to a DNA sequence comprising a heterologous immunoglobin protein-coding sequence, Boss et al. disclose a DNA sequence encoding immunoglobin heavy and light chains which are capable of being expressed and assembled in transformed yeast cells . . . Bruggemann et al. disclose expression of a recombinant chimeric immunoglobin in body fluids, including milk, of transgenic mammals (see, e.g., pages 11-13, under Example 2, Table I, and Figure 5). Bruggeman et al. indicate that transgenic animals can be used for specific antibody production thus allowing large scale production from milk, colostrums, sera, saliva, etc., as well as allowing the breeding of animals that yield a milk that is dosed with specific beneficial antibodies . . . In addition, Weidle et al. disclose a construct comprising DNA sequences encoding immunoglobin heavy and light chains, which are capable of being expressed in transgenic mice, rabbits and pigs harboring the construct . . . From the teachings of Boss et al., Bruggeman et al., and Weilde et al. one of ordinary skill in the art would have had a high expectation of successfully producing a construct comprising a DNA sequence encoding a heterologous immunoglobin sequence which can be expressed and assembled in a host or in the milk of a host mammal.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the DNA sequence disclosed by Meade et al. by substituting the casein promoters or β-lactoglobulin promoter with promoter sequences obtained from the whey acid protein gene or α-lactalbumin gene in view of the teachings of Bischoff et al., Gordon et al., Elbert et al., and Stinnakre et al. that these promoters direct expression of foreign proteins in mammary epithelial cells. It would also have been obvious to substitute the DNA sequence encoding the foreign protein, such as the immunoglobin with the immunoglobin sequences disclosed by Boss et al., Bruggeman et al., or Weilde et al. as hosts transformed with plasmids containing DNA sequences encoding immunoglobulins are capable of synthesizing and secreting the immunoglobulins. Taken together, one of ordinary skill in the art would have had a high expectation of successfully producing heterologous and assembled immunoglobin in the milk of a transgenic mammal which harbors a DNA sequence comprising the coding region of an immunoglobin operatively linked to a promoter which directs expression of a foreign protein in mammary tissue. Thus the claimed invention as a whole was clearly prima facie obvious at the time the claimed invention was

made especially in the absence of sufficient, clear, and convincing evidence to the contrary.

Applicants respectfully traverse this rejection. As discussed above, the claims have been amended to require a unique restriction site between the promoter and the 3'non-coding sequence, wherein the immunoglobulin protein-coding sequence is inserted into the restriction site. The unique restriction site provides a vector that can be modified quickly for expression of a lot of different immunoglobulin protein-coding sequences. Thus, this restriction site adapts to the unique features of expressing immunoglobulins. None of the references cited teach or suggest the use of a unique restriction site nor do the teach or suggest the desirability of such a site to allow for various immunoglobulin-protein coding sequences to be easily inserted into a vector and introduced into a transgenic animal. Thus, the claimed invention is not obvious in view of the cited references. Therefore, Applicants respectfully request that the Examiner withdraw this rejection.

Double Patenting Rejection

Claims 19, 20, and 22-25 are rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over claims 1, 2, and 5 of U.S. Patent No. 5,750,172. According to the Examiner,

> although the conflicting claims are not identical the are not patentably distinct from each other because the expression system comprising a DNA sequence coding for a recombinant polypeptide chain operably linked to a casein promoter, wherein the recombinant polypeptide is selected from immunoglobulins, and wherein the expression system is utilized in generating a transgenic mammal, as claimed in U.S. Patent No. 5,750,172, contains the same DNA sequence components as claimed in the instant application. Moreover, the intended use of the claimed DNA construct in the instant application, i.e., for the generation of a transgenic mammal which expresses the construct in mammary gland epithelial cells and secretes immunoglobulin into the transgenic mammal, is encompassed in the patented claims.

Applicants respectfully traverse this rejection. As amended, the claims of the instant application are directed to a DNA construct for providing a heterologous immunoglobulin in the milk of a transgenic mammal. The construct includes a promoter sequence that results in the

preferential expression of a protein-coding sequence in mammary gland epithelial cells, an immunoglobulin protein-coding sequence, a 3' non-coding sequence, and a unique restriction site between the promoter and the 3'non-coding sequence, wherein the immunoglobulin protein-coding sequence is inserted into the restriction site.

Unlike the instant application, the claims of U.S. Patent No. 5,750,172 do not teach or suggest the claimed construct having a unique restriction site in between the promoter and the 3' untranslated region into which an immunoglobulin protein-encoding sequence is inserted. Thus the claims of the instant application and those of U.S. Patent No. 5,750,172 are patentably distinct. Therefore, Applicants request that the Examiner withdraw this rejection.

Conclusion

In view of the remarks set forth above, it is respectfully submitted that this application is in condition for allowance. If the Examiner believes that a telephone conversation with Applicants' Attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at (617) 542-5070. Please apply any charges not covered, or any credits, to Deposit Account 06-1050.

Respectfully submitted,

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